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CYCLIC HOMOPENTAPEPTIDES 1. ANALOGS OF TUBERACTINOMYCINS AND CAPREOMYCIN WITH ACTIVITY AGAINST VANCOMYCIN-RESISTANT ENTEROCOCCI AND *PASTEURELLA*

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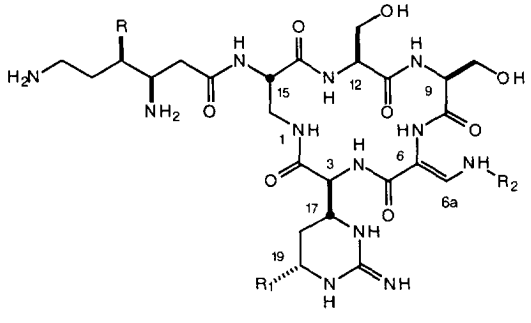
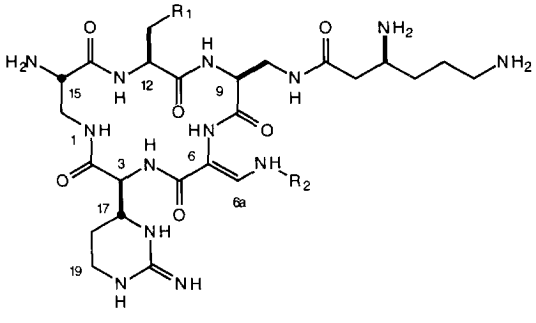
Abstract: A 6a-(3',4'-dichlorophenylamino) analog of viomycin was uncovered by a high-throughput screen against the animal health pathogen *Pasteurella haemolytica*, and has served as a novel lead structure for our infectious disease programs. We report herein the synthesis and activity of analogs of tuberactinomycins and capreomycin that are active against *Pasteurella* spp., methicillin-resistant *Staphylococcus aureus*, and vancomycin-resistant enterococci. © 1997 Elsevier Science Ltd.

Viomycin (tuberactinomycin B), patented by Ciba[1] in 1953 and marketed by both Ciba and Pfizer as a tuberculostatic agent in the 1960s, is a cyclic homopentapeptide containing the unusual amino acids viomycidine, β-lysine, and β-ureidodehydroalanine. A more potent compound, capreomycin, discovered by Lilly[2] in 1960 from fermentations of *Streptomyces capreolus* has a similar structure but is produced as a four-component mixture, with capreomycin IA and IB present as major products, and IIA and IIB as minor ones.[3] In recent years, the biosynthesis of capreomycin has been extensively studied by Gould, et al.[4] Viomycin and capreomycin have potent activity against mycobacteria,[5] with little activity against other genera of bacteria.

Figure 1



tuberactinomycin A; R = OH, R_1 = OH, R_2 = $CONH_2$
tuberactinomycin B (viomycin); R = H, R_1 = OH, R_2 = $CONH_2$
tuberactinomycin N; R = OH, R_1 = H, R_2 = $CONH_2$
tuberactinomycin O; R = H, R_1 = H, R_2 = $CONH_2$

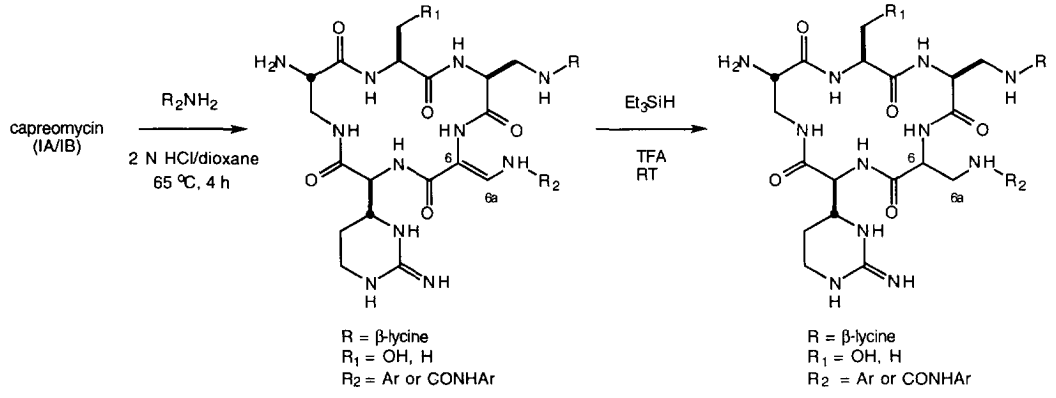


capreomycin IA; R_1 = OH, R_2 = $CONH_2$
capreomycin IB; R_1 = H, R_2 = $CONH_2$
capreomycin IIA; R_1 = OH, R_2 = $CONH_2$ (des-β-lysine)
capreomycin IIB; R_1 = H, R_2 = $CONH_2$ (des-β-lysine)

A 6a-(3',4'-dichlorophenylamino) analog of viomycin, **1**, was uncovered by a high-throughput screen against the animal health pathogen *Pasteurella haemolytica*. This structure has served as a novel lead compound for both animal and human health infectious disease programs. In this communication, we report the conversion of tuberactinomycins and capreomycins, tuberculostatic agents without useful broad spectrum activity, into compounds with promising activity against important gram-negative animal health pathogens such as *P. haemolytica* and *Pasteurella multocida*. These compounds also have activity against several multidrug-resistant gram-positive pathogens found in the hospital environment, including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE).

Chemistry: In the synthesis of over 700 new tuberactinomycin and capreomycin derivatives, the key step was the exchange reaction using various anilines, amines, or phenyl ureas (Scheme 1).[6] A ureido exchange reaction using tuberactinomycin N was reported by Nomoto and Shiba[7] in 1977. After studying the equilibrium of the β-

Scheme 1



ureidodehydroalanine residue in acidic solution, they prepared *N*-methylureido, *N,N*-dimethylureido, and thioureido analogs of tuberactinomycin N by allowing the parent antibiotic to stand for one month at 20 °C with a large excess of the respective urea in 3 N HCl solution. In this work, a more general procedure involving decreased reaction times has been developed. For example, the 6a-(3',4'-dichlorophenylamino)capreomycin derivative **2** (Table 1) was prepared by treatment of capreomycin sulfate (ca. 1:1 ratio of IA:IB) with a 40-fold excess of 3,4-dichloroaniline in 2 N HCl/dioxane at 65 °C for 4 h. The resulting product was purified using a Diaion HP-21 resin,[8] and was characterized by NMR and FAB MS. Phenyl urea analogs could be obtained in a similar manner in 2 N HCl/dioxane solution, but required only 2–10 equiv of the phenyl urea starting material.[9]

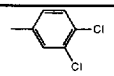
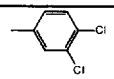
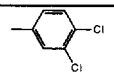
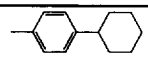
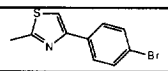
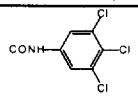
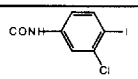
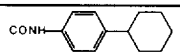
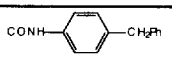
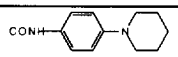
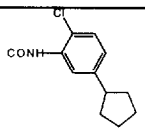
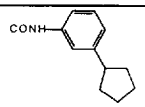
In order to further probe the SAR of the area surrounding C-6, the effect of reducing the double bond was investigated. We discovered that this could be done efficiently and under mild conditions by use of triethylsilane in trifluoroacetic acid (TFA) (Scheme 1).[10,11] The transformation was effected by stirring the substrate in TFA solution with a large excess of triethylsilane at room temperature. The double bond was reduced in the aniline-containing compounds within a few hours, but the more electron-deficient urea-containing compounds took 2-3 days. Interestingly, short-term exposure of viomycin to triethylsilane/TFA allowed isolation of tuberactinomycin O.[12] When these conditions were employed for reduction of viomycin analogs, reduction of the aminal at C-19 and the C-6–C-6a double bond was observed, giving C-6–C-6a dihydro analogs of tuberactinomycin O.

Antibacterial Activity: The in vitro antibacterial activity of some representative compounds (i.e., **1–12**) is illustrated in Table 1. The viomycin derived analog, **1**, afforded modest MICs against a variety of important animal health pathogens including *P. haemolytica*, *P. multocida*, *Actinobacillus pleuropneumoniae*, and *Escherichia coli* (6.25, 0.78, 3.13, and 12.5 μg/mL, respectively). Good in vivo activity has been observed for **1**, with PD_{50} values in mice by subcutaneous (SC) route of administration in the range of 8-10 mg/kg vs. *P. multocida* and *E. coli*.[13] The corresponding analog of **1** in the capreomycin series, **2**, was found to be slightly superior against *P. multocida* both in vitro and in vivo (PD_{50} = 6 mg/kg). Compound **3**, obtained by reduction of the C-6–C-6a double bond of **2**, was somewhat less active in vitro vs. *P. multocida*. The most notable compound in the anilino series was **4**, a 4'-cyclohexylphenylamino analog of capreomycin (PD_{50} = 2 mg/kg in mice vs. *P. multocida*).

As shown in Table 1, compounds with aryl urea side-chains at C-6a also possess in vitro activity against *P. multocida* (i.e., **6-12**). Among these compounds, **6** and **7** also showed good in vivo activity in mice vs. *P. multocida* (PD_{50} = 4 and 7 mg/kg, respectively).

The C-6a substituted capreomycin analogs were significantly more potent against MRSA, and significant nosocomial gram-positive bacterial pathogens, than the parent compound, capreomycin IA/IB (Table 1). The C-6a reduced anilines generally demonstrated decreased potency (e.g., **3** is less active than **2**). Urea-substituted analogs such as **6**, **8**, and **12** displayed good in vitro potency (≤3.12 μg/mL) against these multi-drug resistant clinical strains. The in vitro activity of these analogs against VRE strains was particularly striking as the glycopeptide antibiotic, vancomycin, produced no inhibition at concentrations ≤100 μg/mL. In addition to their demonstrated potency, these compounds were also efficacious in murine infection models. For example, **6** had a PD_{50} (SC) of 3 mg/kg against both MRSA and vancomycin-resistant *E. faecium*.[14]

Table 1. In Vitro Antibacterial Activity, MICs (μg/mL)

Cmpd.	Template (see Fig. 1)	R_2	*P. m.*[15]	*E. coli*[15]	MRSA[16]	*E. f.*[16]	*E. fc.*[16]
—	Cpm IA/IB	$CONH_2$	50	200	100	>1000	>1000
1	Vm		0.78	12.5	12.5	50	25
2	Cpm IA/IB		0.39	6.25	0.78	25	12.5
3	Cpm IA/B; C-6–C-6a dihydro		0.78	6.25	6.25	>100	100
4	Cpm IA/IB		0.2	6.25	1.56	12.5	6.25
5	Cpm IA/IB		3.13	100	12.5	50	12.5
6	Cpm IA		0.2	12.5	0.78	3.12	1.56
7	Cpm IA/IB; C-6–C-6a dihydro		0.78	6.25	3.12	50	12.5
8	Cpm IA		0.39	25	1.56	3.12	1.56
9	Cpm IA/IB		0.78	12.5	1.56	6.25	3.12
10	Cpm IA		1.56	50	3.12	100	100
11	Tum N		NT	NT	3.12	6.25	3.12
12	Cpm IIA		0.39	12.5	3.12	3.12	3.12
—	Vancomycin	—	NT	NT	0.78	>100	>100

Abbreviations: MIC (minimum inhibitory concentration); *P. m.* (*P. multocida*); MRSA (methicillin resistant *S. aureus*: also erythromycin and ciprofloxacin resistant); *E. f.* (*E. faecalis*: vancomycin, methicillin, and ciprofloxacin resistant); *E. fc.* (*E. faecium*: vancomycin, erythromycin, and ciprofloxacin resistant); Cpm (capreomycin); Vm (viomycin); Tum (tuberactinomycin); and NT (not tested).

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References and Notes

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2. Herr, E. B., Jr.; Haney, M. E.; Pittenger, G. E. *Proc. Ind. Acad. Sci.* **1960**, *69*, 134.
3. Stark, W. M.; Boeck, L. D. *Antimicrob. Agents Chemother.* **1965**, 157.
4. (a) Gould, S. J.; Minott, D. A. *J. Org. Chem.* **1992**, *57*, 5214. (b) Wang, M.; Gould, S. J. *J. Org. Chem.* **1993**, *58*, 5176.
5. Herr, E. B., Jr., Redstone, M. O. *Annal. N. Y. Acad. Sci.* **1966**, *135*, 940.
6. In a search for potential tuberculostatic agents, a number of viomycin and capreomycin analogs were first prepared using this exchange reaction with anilines and phenyl ureas at Pfizer Central Research, Sandwich, UK, in the 1960s in a joint collaboration with A. W. Johnson, B. W. Bycroft, and A. Hassanali–Walji of the Univ. of Nottingham, England, and J. D. Hardstone and J. E. Thorpe, Pfizer Ltd; however, purification and characterization were very difficult without modern analytical and spectroscopic methods (private communication with J. D. Hardstone).
7. Nomoto, S.; Shiba, T. *J. Antibiot.* **1977**, *30*, 1008.
8. Mitsubishi Kasei Corp.; a high porous polymer absorption resin that was pre-swollen in methanol for 0.75 to 12 h and rinsed well with water prior to use.
9. Typical procedures using anilines and phenyl ureas are as follows. To a solution of 3,4-dichloroaniline HCl salt (46 g, 0.23 mol) in 60 mL 2 N HCl solution and 60 mL dioxane heated to 65 °C under N_2 atmosphere was added a solution of capreomycin sulfate (5.0 g, 5.8 mmol; IA:IB ca. 50:50; Capastat^R Sulfate, Eli Lilly and Co.) in 20 mL 2 N HCl solution and 20 mL dioxane. The reaction mixture was allowed to stir at 65 °C for 4 h, and then cooled to room temperature. Water (600 mL) was added and the reaction mixture was adjusted to a pH of 8.5 with sodium bicarbonate. The resulting precipitate (excess 3,4-dichloroaniline) was removed by suction filtration and the filtrate was washed with methylene chloride (8X with 500 mL), ether (2X with 300 mL) and hexane (1X with 300 mL). The aqueous layer was adjusted to a pH of 3 with 2 N HCl solution and treated with 250 g of activated Diaion HP-21; the mixture was stirred with an overhead paddle stirrer for 2 h. The resin was removed by suction filtration and washed with water (4X with 1 L;

which eluted unreacted antibiotic starting material) and then methanol (4X with 500 mL). The methanol washes were combined and evaporated under vacuum to a volume of about 5 mL. The addition of a small amount of methanol resulted in precipitation of 4.7 g (the salt contained 75% active material based on C,H,N analysis; 75 % corrected yield) of the desired product as an HCl salt. The positive FAB MS of **2** gave diagnostic cationized molecules at *m/z* 770 (M+1) for the IA analog, and at *m/z* 754 (M+1) for the IB analog. The syntheses of corresponding phenyl urea analogs were performed in a similar manner in 2 N HCl/dioxane solution, but required only 2–10 equiv of the phenyl urea starting material. These reactions were typically heated at 65 °C overnight, and then allowed to cool to ambient temperature. The precipitated urea was filtered off and the mixture was poured into water. The aqueous layer was then washed 4X with ethyl acetate before pouring onto activated HP-21 resin in a beaker, covered with a watch glass, and gently shaken (orbital shaker) for 7 h. The resin was filtered (coarse frit), rinsed thoroughly with water (5X), and transferred into a beaker before adding methanol:water (in a 9:1 ratio), shaking for 3 h, and pouring through a frit to collect the extract. After solvent removal the sample was lyophilized to yield the desired product (ca. 60–80% yield).

10. Magnus, P.; Gallagher, T.; Schultz, J.; Or, Y.-S.; Ananthanarayan, T. P. *J. Am. Chem. Soc.* **1987**, *109*, 2706.
11. Reduction of the C-19 OH group of viomycin by catalytic hydrogenation is accompanied by formation of a C-6 methyl derivative (with loss of the urea moiety): Kitagawa, T.; Miura, T.; Tanaka, S.; Taniyama, H. *J. Antibiot.* **1973**, *26*, 528.
12. Tuberactinomycin O is produced as a minor component in a mixture of fermentation products; thus, the present semisynthetic procedure allows easy entry to this antibiotic: Izumi, R.; Noda, T.; Take, T.; Nagata, A. *J. Antibiot.* **1972**, *25*, 201.
13. The gram-negative acute murine infection models were conducted in a manner described previously: Dirlam; J. P.; Presslitz, J. E.; Williams, B. J. *J. Med. Chem.* **1983**, *26*, 1122.
14. The gram-positive acute murine infection models were performed in a manner described previously: Girard, A. E.; Girard, D.; Gootz, T. D., Faiella, J. A., Cimochowski, C. R. *Antimicrob. Agents Chemother.* **1995**, *39*, 2210; additional in vivo data for these compounds will be provided in a full paper.
15. MICs against *P. multocida* and *E. coli* were analyzed by a microdilution method similar to that described in the NCCLS document M7-A3 (Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, 3rd Edition, Villanova, PA, 1993), with the exception that the total volume of the well was 200 μL instead of 100 μL, and BHI (brain-heart infusion) broth was used in place of CAMH broth.
16. MICs against *E. faecalis* and *E. faecium* were analyzed according to the procedures described in NCCLS document M7-A3, cited in ref 15.

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